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Isolation and identification of wheat gene *TaDIS1* encoding a RING finger domain protein, which negatively regulates drought stress tolerance in transgenic *Arabidopsis*

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ABSTRACT

Drought stress is a major factor that limits the yield and quality in wheat. In this study, we identified an orthologue of the rice gene *OsDIS1* (*Oryza sativa* drought-induced SINA protein 1) in wheat (*Triticum aestivum* L.) called *TaDIS1*. *TaDIS1* encodes a putative 301 amino acid protein with a C3HC4 RING finger conserved domain at the N-terminal and a SINA domain at the C-terminal. *TaDIS1* contains three exons and two introns. qRT-PCR analysis showed that *TaDIS1* expression was induced by PEG6000, NaCl, and abscisic acid (ABA) treatment. We generated *TaDIS1*-overexpressing transgenic *Arabidopsis* lines. Under drought stress conditions, the transgenic *Arabidopsis* plants had a lower germination rate, relative water content, and proline contents, with higher water loss, chlorophyll loss, relative electrical conductivity, and malondialdehyde contents compared with the wild type. The antioxidant enzyme (superoxide dismutase, peroxidase, and catalase) activity levels were lower in the transgenic plants. The *TaDIS1*-overexpressing plants had shorter roots with greater growth inhibition in response to mannitol treatment than the wild type, with increased hypersensitivity to ABA during seed germination and early seedling growth. The expression of stress-related genes in transgenic plants under drought stress suggests that *TaDIS1* may function negatively in drought stress by regulating the stress response-related genes.

1. Introduction

Plants are subjected to a wide variety of abiotic stresses, which have many severe adverse effects on plant growth and development. Abiotic stresses are responsible for global yield reductions of over 50% in the major crop plants [1]. Thus, in order to survive and reproduce better, plants must develop effective mechanisms to adapt to stressful environments [2]. Plants can modulate their phenotypes according to changes in physiological, biochemical, molecular, and genetic information, thereby allowing them to tolerate abiotic stresses [3]. Many abiotic stress-induced genes have been identified in previous studies of various plants, and their roles in stress responses have been well documented [4–7]. Numerous studies have also shown that plants can acquire tolerance to abiotic stress by re-regulating their metabolism and gene expression [8–10]. Therefore, isolating and cloning the key stress resistance genes is important for the development of new wheat varieties via conventional breeding and genetic engineering strategies [11].

The ubiquitin/26S proteasome system is one of the main mechanisms employed by plants to control their growth and development, as well as for responding to biotic and abiotic stresses [12–15]. E3 ligase is the most important enzyme among the ubiquitin-mediated protein degradation pathways [16]. In the RING E3 ligase group, RING finger proteins are known to play important roles in responses to abiotic stresses [17]. Some progress has also been made in the cloning and functional characterization of RING domain type E3 ubiquitin ligase-related genes, where their functions in the response to drought stress have been elucidated in *Arabidopsis*. For example, C3H2C3-type RING E3 ubiquitin ligase AtAIRP1 in *Arabidopsis* is a positive regulator of the abscisic acid (ABA)-dependent response to drought stress [18]. *Arabidopsis RGLG2* encodes a RING E3 ligase, which can interact with *AtERF53* and it negatively regulates the drought stress response by mediating the transcriptional activity of *AtERF53* in *Arabidopsis* [19]. *Arabidopsis* C3HC4-RING finger E3 ubiquitin ligase AtAIRP4 may act as a positive regulator of ABA-mediated drought avoidance and as a negative regulator of salt tolerance [20]. In rice, many RING finger proteins are also involved with the responses and adaptations to abiotic stress. According to previous studies, the overexpression of *Oryza sativa* RING domain-containing protein 1 (*OsRDCP1*) and *O. sativa* chloroplast

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Table 1
Gene-specific primers used in this study.

Gene	Upstream primer (5′-3′)	Downstream primer (5′-3′)
TaDIS1-P1	ACTGACTATGAGTTCACTGGCAATC	GACGATGGCTAAAACAGGCTAC
TaDIS1-P2	GGCCTCGGCTGCTTATACTG	AGCAGACAGGGCATTCCAAA
18SrRNA	AACACTTCACCGGACCATTCA	CGTCCCTGCCCTTTGTACAC
TaDIS1-P3	TCGGTACCCTCGAGGGATCCATGGCCTCGGCTGCTTATA	GACTGCAGGTCGACAAGCTTCTCTTCTTTCCAAATCCTCCC
TaDIS1-P4	GGACTCTTGACCATGGCCTCGGCTGCTTATACTG	TCAGATCTACCATGGCCTCTTCTTTCCAAATCCTCCC
AtActin	GGTAACATTGTGCTCAGTGGTGG	AACGACCTTAATCTTCATGCTGC
DREB2 A	CTGGAGAATGGTGCGGAAGA	CAGATAGCGAATCCTGCTGTTGT
RD29A	GTTACTGATCCCACCAAAGAAGA	GGAGACTCATCAGTCACTTCCA
RD29B	GGAGTTCAAGATTCTGGGAAC	CATCAAAGTTCACAAACAGAGGC
P5CS1	GCGCATAGTTTCTGATGCAA	TGCAACTTCGTGATCCTCTG

targeting RING E3 ligase 1 (*OsCTR1*) improve drought tolerance in transgenic plants [21,22]. The RING finger E3 ligase gene *OsDSG1* controls seed germination and stress responses in rice [23]. The *OsSDIR1* gene is a functional orthologue of *Arabidopsis SDIR1* and it encodes a functional E3 ligase. The overexpression of *OsSDIR1* greatly improves drought tolerance in transgenic rice [24].

OsDIS1 (*Oryza sativa* drought-induced SINA protein 1) encodes a C3HC4 RING finger E3 ligase. The expression of *OsDIS1* is upregulated by drought treatment. The overexpression of *OsDIS1* reduces drought tolerance in transgenic rice plants and RNA interference (RNAi) to silence *OsDIS1* enhances drought tolerance. Microarray analysis has shown that a large number of drought-responsive genes are induced or suppressed in *OsDIS1* overexpressing plants under normal and drought conditions. Alternatively, RNAi transgenic plants with reduced *OsDIS1* expression levels can be generated for rice production if transgenic rice cultivars are allowed to grow in the field [25].

Most of the previously characterized RING finger type E3 ligases were identified in *Arabidopsis* or rice, and little is known about the RING E3 ligases in wheat. In our investigations of new genes involved with wheat defense responses, we isolated the *TaDIS1* gene based on homologous cloning. *TaDIS1* encodes a C3HC4 RING finger protein. We investigated the expression patterns of *TaDIS1* and its response to stress stimuli using quantitative real-time PCR (qRT-PCR), and we studied its functions in drought tolerance by overexpressing *TaDIS1* in *Arabidopsis* plants. Phenotypic analyses indicated that the overexpression of *TaDIS1* decreased drought tolerance in transgenic *Arabidopsis*, which exhibited an ABA hypersensitive response. These findings suggest that *TaDIS1* might participate in the negative regulation of drought stress tolerance in wheat plants.

2. Materials and methods

2.1. Plant material and stress treatment

Bread wheat (*Triticum aestivum* L. cv. Chinese Spring) was used in this study. The seeds were surface sterilized with 1% sodium hypochlorite for 15 min and rinsed three times with distilled water. The sterilized seeds were then grown in Petri plates containing two layers of filter paper wetted with distilled water in a growth chamber at 22 °C. Ten-day-old wheat seedlings were subjected to various abiotic stresses. In order to mimic drought, salinity, and ABA stress treatments, seedlings were transferred into solutions containing 20% PEG6000, 200 mM NaCl, and 100 μM ABA, respectively. Leaves were collected at 0, 3, 6, 9, 12, and 24 h after various treatments for gene expression analysis. Harvested plants were dropped immediately into liquid nitrogen and stored at −80 °C until RNA extraction. Wheat seeds were sown in the field to obtain different tissues from the growing wheat plants. Conventional agricultural management was maintained during growth and development. Different tissues were collected from wheat plants at the booting stage to determine tissue-specific expression levels and the samples were stored at −80 °C until use.

2.2. Isolation of the TaDIS1 gene

The complete cDNA sequence of the rice *OsDIS1* gene [25] was obtained from GenBank by searching with the GenBank accession (AK058336). The rice sequence was used to BLAST against the wheat genomic database at Unite de Recherche Genomique Info (URGI; https://urgi.versailles.inra.fr/blast/). Many genomic sequences shared high identity with *OsDIS1* and they were assembled into integrated contig sequences. A pair of gene-specific primers was designed to cover the open reading frame (ORF) sequence based on the assembled contig sequence in order to amplify the full-length *TaDIS1* cDNA sequence. Total RNA was isolated from the young leaves of Chinese Spring plants using TRIzol reagent according to the manufacturer's instruction (TaKaRa, China). First-strand cDNA was synthesized using a PrimeScript RT Reagent Kit with gDNA Eraser (Tiangen, Beijing, China) according to the manufacturer's recommended protocol. PCR was performed using the cDNA as the template with the primer *TaDIS1*-P1 (Table 1). The PCR products were purified and cloned into the pEASY-T1 vector and confirmed by sequencing.

The genomic DNA sequence was cloned to further analyze the structure of the *TaDIS1* gene. Genomic DNA was extracted from the young leaves of field-grown Chinese Spring plants using a modified CTAB method [26]. We used 1 μ of the genomic DNA as a template for PCR amplification with the primer *TaDIS1*-P1. The PCR products were purified and cloned into the pEASY-T1 vector and confirmed by sequencing.

2.3. Bioinformatics analysis

The sequence obtained was spliced using DNAMAN software to obtain the full length of the target gene. The protein encoded by the target cDNA was predicted based on the amino acid sequence and BLAST was performed via the NCBI website to analyze the sequences of the ORFs and conserved domains. Alignments were obtained with ClustalX (version 1.83) and GeneDoc software. A phylogenetic tree was constructed using ClustalX and MEGA6.0 (http://www.megasoftware.net/mega.html). The basic physical and chemical properties of the TaDIS1 protein were predicted using the Protparam tool (http://www.expasy.org/tools/protparam.html). The grand average of hydropathy (GRAVY) was predicted for the TaDIS1 protein using the Hplob./Kyte & Doolittle algorithm in ProtScale online software. The secondary structure of the *TaDIS1* protein was predicted using SOPMA (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_sopma.html). The conserved domains were analyzed using the PROSITE online database (http://expasv.org/prosite/).

2.4. qRT-PCR analysis of TaDIS1 expression patterns

Total RNA was extracted from different organs and various stress-treated materials (see above) using Trizol according to the manufacturer's instructions (Tiangen, Beijing, China), before it was reverse